Acute stress enhances intestinal permeability to intact protein in Roman Low Avoidance rats, but not in Roman High Avoidance rats, by a cholinergic mechanism.

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ACUTE STRESS ENHANCES INTESTINAL PERMEABILITY TO INTACT PROTEIN IN ROMAN LOW AVOIDANCE RATS, BUT NOT IN ROMAN HIGH AVOIDANCE RATS, BY A CHOLINERGIC MECHANISM. P.B. Bijlsma, P.R. Saunders*, P. Driscoll**, J.M. Koolhaas***, J.A.J.M. Taminiau, M.H. Perdue*, J.A. Groot. Inst. Neurobiol / Acad. Med. Ctr., Univ. Amsterdam,*** Fac. Biol., Univ. Groningen, The Netherlands; *Dept. Pathol., McMaster Univ. Hamilton, Canada; **ETH Zürich, Switzerland.

Previously we have shown an enhanced intestinal macromolecular permeability after acute cold restraint stress in the stress-susceptible rat strain Wistar Kyoto, which occurred via a cholinergic mechanism (Gastroenterol. 108 Suppl: A911, 1995). Here we examined the effect of cold restraint stress in Roman High Avoidance (RHA) rats which have an active, mainly sympathic stress coping style, and in Roman Low Avoidance (RLA) rats, which have a passive, mainly parasympathic stress coping style. Rats were stressed by immobilization for 2 hours at 8° C followed by 2 hours recovery at room temperature in their home cage. Atropine i. p. (2mg/kg) was given to 50% of the rats prior to restraint, or 4 hours prior to sacrifice in controls.
Permeability of stripped segments of jejunum to horseradish peroxidase (HRP, 40kD, 10^{-5} M added to the mucosal side, serosal appearance measured enzymatically) was studied in Ussing chambers in carbogenated Ringer's solutions at 37°C. Mean one hour HRP accumulations ± s.e.m. (in pmol/cm^2) are given in the table. Significant differences from controls were tested by two-tailed t-test (*$p < 0.02$).

	contr	*stress*	*contr atrop*	*stress atrop*
RHA	2.0 ± 0.6	2.0 ± 0.5	3.0 ± 0.6	1.4 ± 0.2
n	5	6	5	5
RLA	0.9 ± 0.2	3.5 ± 0.9*	1.6 ± 0.3	1.1 ± 0.5
n	5	6	6	6

Acute cold restraint stress caused a significant, more then 3-fold increase in intact HRP permeability in RLA rat jejunum, which could be blocked by prior injection of rats with atropine. This is consistent with the primarily parasympatic (vagal) stress response in RLA rats, and indicates the involvement of cholinergic activation in the observed permeability increase. In RHA rats a stress-induced increase in jejunal HRP permeability was not apparent. Injection of atropine in both RHA and RLA controls tended to increase intestinal HRP permeability, which may indicate that in control rats of the Roman strain a minimal cholinergic tone is required to maintain intestinal barrier function, while in stressed animals atropine may reduce an increased cholinergic tone back to control levels. We conclude that effects of stress on intestinal barrier function may be dependent on individual stress coping strategies. This may be of relevance for patients with inflammatory bowel disease, in which symptoms are often aggravated by stressful events.
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● G1435

SUBCHRONIC MILD ACOUSTIC STRESS ENHANCES THE *IN VITRO* SMALL INTESTINAL PERMEABILITY TO INTACT PROTEIN IN RATS BY INCREASED ENDOCYTIC TRANSPORT. P.B. Bijlsma, *M.T.M. van Raaij, *C.J.G. Dobbe, *A. Timmerman, A.J. Kiliaan, J.A.J.M. Taminiau, J.A. Groot. Fac. Biol./Acad. Med. Ctr, Univ. of Amsterdam; *Nat. Inst. of Public Health and Environment, Bilthoven, The Netherlands.

Recently we reported an increased trans- and para-cellular protein permeability in rat small intestine after acute (2 hours) cold restraint stress (Gastroenterol. 108 Suppl: A911, 1995). This is a rather strong stressor, as judged from the observed 225 -250 ng/ml increment in plasma corticosterone levels (Neuroendocrinol. 65: 200-209, 1997; Am. J. Physiol. 267: G794-G799, 1995). In the present study we applied randomized 95 dB white noise pulses during 45 min per hour, 12 hours per day, duration 8 days, as a mild subchronic stressor to male Wistar rats (± 250 g). At 8 days (day -8) before the noise experiments (day 0), 50% of the animals were cannulated in the vena cava allowing free movement, and blood samples were obtained at day -1, 0, 1, 2, 4, 7 and 9. The other 50% of the animals were sacrificed at day 9, segments of ileum were stripped from muscle layers and mounted in Ussing chambers and perfused in carbogenated Ringer's solutions of 37° C. Horse Radish Peroxidase (HRP, 40kD, 10^{-5} M) was added to the mucosal side, and serosal samples were taken at 60 and 120 minutes. Tissues were fixated for electronmicroscopical HRP staining and serosal appearance of HRP was detected enzymatically. In the cannulated noise-exposed animals, serum catecholamine and prolactine levels were unchanged, corticosterone levels were significantly enhanced from day 2 compared to controls (36 ± 11 vs 16 ± 5 ng/ml, $p < 0.01$, Mann-Witney test), and remained at this level at day 7 and 9. Ileal HRP-flux (in pmol/cm^2.h) was significantly enhanced in noise-exposed animals (n=12) compared to controls (n=13), resp. 2.3 ± 0.4 vs 1.0 ± 0.2 at 0 - 60 minutes, and 5.9 ± 0.8 vs 3.3 ± 0.5 at 60 - 120 minutes, $p < 0.01$. Electronmicrographs of tissue from stressed or control animals showed no clear traces of paracellular HRP-staining. Quantification of HRP-containing endosomes in enterocytes revealed a significant increase in endosome number (0.77 ± 0.16 vs 0.38 ± 0.15, per apical area of 4 x 6 µm, $P < 0.04$). Moreover, there was a positive correlation between the number of HRP-filled endosomes and HRP flux in both control ($r=0.82$, $p < 0.005$) and stressed animals ($r=0.65$, $p < 0.05$), with similar slopes and y-axis offsets of the correlation lines, indicating that the increased HRP permeability was primarily due to increased endocytosis. We conclude that in addition to strong acute cold restraint stress, also mild subchronic noise stress may cause a decrease in intestinal barrier function by increased transcytosis of luminal antigens, although with no clear involvement of paracellular leak. The twofold increase in macromolecular permeability may lead to an increased antigenic load to the mucosal immune system, thus possibly stimulating sensitization to food antigens and inflammatory responses of the gut mucosa.
This research was supported in part by a grant from Nutricia, Zoetermeer, The Netherlands

● G1436

TISSUE-SPECIFIC, DIFFERENTIAL AND PRETRANSLATIONAL REGULATION OF NA-H EXCHANGE ISOFORMS BY ALDOSTERONE IN RAT COLON. H.J. Binder, M. Ikuma, V.M. Rajendran. Department of Internal Medicine, Yale University, New Haven, CT.

Aldosterone (aldo) has multiple, diverse effects on ion transport in rat colon including both induction and inhibition of electroneutral Na-Cl absorption and Na-H exchange (NHE) in proximal and distal colon, respectively. The mechanisms of these divergent effects are poorly understood. To determine the mechanism of the differential regulation of Na transport by aldo, we performed: 1) [H] gradient-driven ^{22}Na uptake by apical membrane vesicles (AMV) in presence of 1 and 25 µM HOE694, an amiloride analogue, with dose-dependent specificity for NHE-1, NHE-2 and NHE-3 isoforms (NHE-1 is inhibited by 1 µM, NHE-2 by 25 µM, while NHE-3 is not affected by 25 µM HOE694); and 2) Northern blot analyses of NHE isoformspecific mRNA abundance. HOE694 inhibition studies of ^{22}Na uptake in proximal colon demonstrated that aldo did not alter NHE-1, but increased NHE-2 and NHE-3 by 7.6 fold and 2.0 fold, respectively, as a result of an increase in Vmax (4.4 ± 0.4 vs 16.6 ± 1.2 nmol/mg prot.6 sec) without a change in Km for Na. Northern blot revealed an increase in NHE-2 (190%), NHE-3 (300%) mRNA but no change in NHE-1 mRNA abundance. In contrast, aldo in distal colon abolished both NHE-2 and NHE-3 function in ^{22}Na uptake studies, while resulting in almost complete inhibition in NHE-2, NHE-3 but not NHE-1 mRNA abundance. **Conclusion:** Aldo increases both NHE-2 and NHE-3 function and message in proximal colon but inhibits both NHE-2 and NHE-3 function and message in distal colon. The molecular mechanisms for this tissue-specific, differential, pretranslational regulation of NHE function remain obscure.

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INCREASED TENSION GENERATION BY INTESTINAL SMOOTH MUSCLE IS MEDIATED IN PART BY LEUKOTRIENES AND PROSTAGLANDINS. P.A. Blennerhassett, B.A. Vallance and S.M. Collins, GI Division and the Intestinal Diseases Research Program, McMaster University, Ontario, Canada, L8N 3Z5

We have shown that infection of mice with the nematode Trichinella spiralis is accompanied by increased tension generation by jejunal longitudinal muscle. Our studies have shown that this increase is mediated via the inflammatory response to the infection and that CD4+ lymphocytes contribute to the changes. The role of other cell types and their mediators remains to be determined. In this study, we have examined the roles of prostaglandins and leukotrienes as putative mediators of these changes.
129SV mice were infected with 375 T.spiralis larvae and sacrificed day 8 post infection. Uninfected 129SV mice were used as controls. Tissues were frozen in liquid nitrogen for analysis of prostaglandin (PG) and leukotriene (LT) concentrations, and for myoloperoxidase(MPO) activity as a measurement of the acute inflammatory response. Full thickness segments of jejunum were suspended in organ baths at 37C containing Kreb's buffer at optimum tension and allowed to equilibrate. The tissue were stimulated with carbachol (10-5M) and isometric contraction measured. The tissues were then exposed to indomethacin (1µM) or MK-886 (1µM) for two hours before retesting the response to carbachol.
Infection was accompanied by a 4-fold increase in MPO activity, a 5-fold increase in PGE_2 and a 4-fold increase in both LTB_4 and C_4. These increases in inflammatory mediators were accompanied by a 200% and a 300% increase in muscle tension generated by carbachol and KCl respectively. Inhibition of 5' lipoxygenase using MK-886 had no effect on muscle from control mice but further reduced the contractile response to carbachol or KCl by 25% and 70% respectively. Blockade of arachidonate metabolism by indomethacin did not influence muscle from control mice but reduced the responses to carbachol by 20 and 60% respectively in *T.spiralis* infected mice.
These data indicate that primary nematode infection in mice results in an increase in PGE_2 and in LTB_4 and C_4 concentrations in the intestine. These agents in turn contribute to the increase in force generation by intestinal muscle in this model.
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